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(54) PHARMACEUTICAL COMPOSITION CONTAINING TRIMETHAPRIN, SULPHAMETHOXAZOLE AND ALPHA-AMYLASE

(71) We, PARCOR, a French corporate body of 60 rue de Wattignies, 75012 Paris, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

The present invention relates to a pharmaceutical composition having, in particular, antibacterial activity and containing as active principle a mixture of trimethoprim, a sulphonamide and α -amylase.

Diaminopyrimidines having antibacterial activity are known from the work of Hitchings et al. (J. Biol. Chem., 1948, 174, 765—766). Of these products, trimethoprim, i.e. 2,4 - diamino - 5 - (3,4,5 - trimethoxybenzyl) - pyrimidine was chosen for its good tolerance and anti-infectious activity, which are also well known (Roth et al., J. Mednl. Pharm. Chem. 1962, 5, 1103—1123).

It has also been observed that trimethoprim has a potentiating effect on sulphonamides (Elion et al., J. Biol. Chem., 1954, 208, 477—488) and particularly on sulphamethoxazole, i.e. 5 - methyl - 3 - sulphonylamido - isoxazole.

Trimethoprim and sulphamethoxazole have been used in admixture before and the antibacterial activity of this mixture is described in the literature (Pechere et al., Thérapie, 1970, 25, 13—28).

This invention provides a pharmaceutical composition comprising trimethoprim, sulphamethoxazole and α -amylase. The presence of this enzyme in the composition has in our tests surprisingly shown results which are clearly superior to those conventionally obtained with binary mixture of trimethoprim and sulphamethoxazole. The amylase may be of vegetable, bacterial or animal origin or be extracted from fungi; it is a soluble enzyme conventionally used in therapy, to aid the digestion of starchy substances, or as anti-dyspeptic in amylaceous dyspepsia and hyper-

acidic dyspepsia. Recent research has shown that α -amylase also has valuable anti-inflammatory and anti-oedematous properties. Other tests have shown that, in animals, α -amylase ensures more widespread diffusion of antibiotics of the tetracycline type in the organism (Comm. Soc. Pharm. Toulouse, 5th December 1973).

In general, the compositions may contain from 10 to 1,000 parts by weight of trimethoprim together with 100 to 10,000 parts by weight of sulphamethoxazole per 1,000 to 100,000 units of α -amylase. The α -amylase unit is the quantity of enzyme which destroys 1 milligram of soluble starch at 37°C in 100 seconds.

The active constituents may be admixed with a physiologically acceptable carrier.

The compositions of the invention have very valuable therapeutic properties which result from a remarkable synergistic action between the components of the active mixture.

The compositions are usually presented for administration by oral route in the form of tablets, coated tablets, lacquered tablets, gelatine capsules or powders. The compositions may be presented in dosage unit form, each dosage unit containing for example 0.020—1 g of trimethoprim 0.020—5 g of sulphamethoxazole and 500—60,000 units of α -amylase.

The unexpected affects of the compositions of the invention were studied in comparison with the bactericidal properties of the known mixture of trimethoprim and sulphamethoxazole. In order to do this, an investigation was made to determine the synergistic effect of the compositions of the invention in infectious pathology, and they were shown to have a bactericidal effect superior to that of the known mixture of sulphamethoxazole and trimethoprim.

The tests proving the particular characteristics of the compositions according to the

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invention was carried out on different microbial strains from a hospital under experimental conditions which are described in detail hereinafter.

5 1. *Apparatus and methods*

1.1) The study was carried out on many microbial strains. Two tested strains, *Escherichia coli* K12 and *Staphylococcus aureus* 209P, as well as other microbial strains tested were taken from hospital patients. Only the results of tests against pathological strains are reported below.

1.2) The composition of the invention was dissolved in distilled water in a ratio of 10mg of trimethoprim, 50mg of sulphamethoxazole and 400 units of α -amylase.

1.3) The synthetic culture medium used was the one described by Adams & Roe (J. Bact., 1945, 49, 401-409).

1.4) *Inoculum*: each microbial strain being tested was seeded in the above mentioned medium and after 24 hours' growth dilution was effected to give an inoculum of approximately 10^5 organisms/ml.

1.5) Preparation of tubes and count:

Each haemolysis tube contained 0.4 ml of the Adams & Roe medium, 0.1ml of inoculum and 0.5ml of the solution to be tested. After 18 hours' incubation at 37°C, a count was made of the surviving organisms; this count was made by seeding 1/10 ml quantities in a

Petri dish containing soya gelose trypticase.

1.6) Study of the bactericidal power of the medicament of the invention:

25 tubes are seeded for each strain being studied. Tube no. 1 is a control and does not contain any antibacterial compounds. Tubes 2 to 9 contain a mixture of trimethoprim and sulphamethoxazole in a ratio of 1:5, the concentration of the mixture ranging from 1 to 128 μ g/ml (i.e. tube 2 contains 1 μ g/ml of the mixture, tube 3 contains 2 μ g/ml, tube 4 contains 4 μ g/ml, tube 5 contains 8 μ g/ml, tube 6 contains 16 μ g/ml, tube 7 contains 32 μ g/ml, tube 8 contains 64 μ g/ml and tube 9 contains 128 μ g/ml). Tubes 10 to 17 contain the composition of the invention in the proportions defined in paragraph (1.2) above, the concentration (of the trimethoprim and sulphamethoxazole) being varied over the series of tubes in exactly the same way as given above for tubes 2-9. Tubes 18 to 25 contain α -amylase in a concentration of 0.044 units per ml.

2. *Results obtained*

The results below are given only as illustration. Thus, taking into account only the percentage of organisms surviving under the above-mentioned conditions. The results obtained are summarised in the following Table A:

Organisms	α -amylase 0.044 U	Trimethoprim + Sulphamethoxazole 1/5 (μ g/ml)	Trimethoprim + Sulphamethoxazole + α -amylase 1/5 μ g/ml 0.04 U α -amylase ml
Klebsiella	99%	0.1% survivors at concentration 4	0.03% survivors at concentration 4
Salmonella bovis mortificaus	92.7%	12% survivors at concentration 2	1% survivors at concentration 2
Bordetella bronchiseptica	100%	8% survivors at concentration 8	1.5% survivors at concentration 8
Enterobacter	97 %	3% survivors at concentration 1	0.9% survivors at concentration 1

The concentration numbers given in this table refer to the range of concentrations of the tubes 2-9 and 10-17. Thus, concentration 1 contains 1 μ g/ml of the mixture of trimethoprim and sulphamethoxazole; concentration 2 contains 2 μ g/ml; concentration 4 contains 8 μ g/ml; and concentration 8 contains 128 μ g/ml.

There is virtually no bactericidal effect with α -amylase on its own.

The bactericidal effect is incomplete with the mixture of trimethoprim and sulphamethoxazole alone. In contrast, the composition of the invention shows a potentiated

effect and in every case leads to constantly superior bactericidal results. The synergism effected by amylase in the medicament of the invention makes it possible to increase the known bactericidal power of the mixture of trimethoprim and sulphamethoxazole.

Other tests were carried out *in vivo*. Mice of the Swiss strain, with a body weight ranging from 10 to 20 grams, were given an injection of *Proteus vulgaris* culture by the intraperitoneal route. The composition of the invention was administered by gastric tube, in suspension in a 1% aqueous carboxymethyl-cellulose solution, in a volume of 1ml; the first dose administered was given 5 minutes after the intraperitoneal infecting injection and the treatment was continued once a day for 4 days. The animals were kept under observation for 14 days. The number of animals surviving after this period was noted.

The results obtained are given hereinafter, wherein the test is compared with results given by the known trimethoprim/sulphamethoxazole mixture and by amylase alone.

A study of the results shown in Table B demonstrates, in this test *in vivo*, that the activity of the compositions of the invention is clearly superior to that of the known trimethoprim/sulphamethoxazole mixture. Thus it appears that the components of the compositions of the invention show synergism, with one another and the presence of α -amylase in the composition yields unexpected results and effects.

TABLE B

Alpha Amylase		Trime thoprim (T)+Sulpha-methoxazole (S) Dosages administered/kg			Trime thoprim (T)+Sulphamethoxazole (S)+Alpha-Amylase (A) Dosages administered/kg			
Dosages/kg	Survivors	T	S	Survivors	T	S	A	Survivors
500U	0/8	50mg	20mg	2/8	50mg	20mg	500U	4/8
1000U	0/8	50mg	50mg	3/8	50mg	50mg	1,000U	6/8
1500U	0/8	50mg	100mg	3/8	50mg	100mg	1,500U	7/8

30 The following Examples illustrate compositions of the invention.

wax, carnauba wax (q.s. for 1 coated tablet weighing 0.750 g.

Example 1.

TABLETS

Trimethoprim	0.080 g
Sulphamethoxazole	0.350 g
α -amylase	3000 U

Excipient (corn starch, lactose, gelatine, talc, magnesium stearate) q.s. for 1 tablet weighing 0.700 g.

Example 2.

CHILDREN'S TABLETS

Trimethoprim	0.020 g
Sulphamethoxazole	0.100 g
α -amylase	1500 U

40 45 Example 2. Excipient (polyethylene glycol 600, potato starch, carboxypolymethylene, magnesium stearate, talc) q.s. for 1 tablet weighing 0.320 g.

Example 4.
LACQUERED TABLETS

Trimethoprim	0.040 g
Sulphamethoxazole	0.200 g
α -amylase	2000 U

Excipient (lactose, polyvinylpyrrolidone, corn starch, talc, magnesium stearate, sucrose, shellac, castor oil, propylene glycol, indigotin, titanium oxide, white wax) q.s. for 1 coated tablet weighing 0.400 g.

Example 5.

POWDER	
Trimethoprim	1 g
Sulphamethoxazole	5 g
α -amylase	60,000 U

40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 1235 1240 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— septicaemia with various organisms,
— generalised infections such as, e.g.,
those caused by *Proteus* and *staphylococci*.
5 The compositions are contra-indicated in
subjects who are allergic to sulphonamides.

WHAT WE CLAIM IS:—

10 1. A pharmaceutical composition comprising trimethoprim, sulphamethoxazole, and α -amylase.
2. A composition as claimed in claim 1, comprising a physiologically acceptable carrier.
15 3. A composition as claimed in claim 1 or claim 2 comprising 10 to 1000 parts by weight of trimethoprim and 100 to 10,000 parts by weight of sulphamethoxazole per 1000 to 100,000 units of α -amylase.

4. A composition as claimed in any one of the preceding claims in a form suitable for oral administration. 20

5. A composition as claimed in any one of the preceding claims in dosage unit form, each dosage unit containing 0.020—1 g of trimethoprim, 0.020—5 g of sulphamethoxazole and 500—60,000 units of α -amylase. 25

6. A pharmaceutical composition substantially as described herein in any of the Examples.

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